

Significantly reduced expression of immunoproteasome components and their regulators was observed to be associated with epithelial to mesenchymal transition in 42 NSCLC cell lines using proteomic profiling and microarray analysis. A highly variable immunoproteasome expression was also observed among NSCLC tissues. Immunohistochemistry data revealed loss of immunoproteasome subunit is significantly correlated with expression of CDH2 and concomitant loss of CDH1 in NSCLC tumors. Loss of immunoproteasome subunits was also significantly associated with advanced stage ($p=0.014$), recurrence ($p=0.002$) and metastasis ($p<0.01$) in NSCLC patients. A significantly reduced antigen presentation was observed in mesenchymal cell lines compared to epithelial cells. Using mild acid elution, only 50-60 HLA class I bound peptides were identified in mesenchymal cell lines compared to 400-500 peptides in epithelial counterparts. IFN γ as well as 5-aza-2'-deoxycytidine treatment was able to revive immunoproteasome expression and hence restored repertoire of HLA class I bound peptides in deficient mesenchymal cells. Induced expression of immunoproteasome and hence HLA class I bound peptides also lead to significantly enhanced CD8 $^{+}$ T cell mediated cytotoxicity ($p<0.01$) in mesenchymal cells compared to non-induced controls. Our findings point towards a mechanism of immune evasion of cells with a mesenchymal phenotype and strategies to overcome their immune evasion through induction of the immunoproteasome or targeting the limited repertoire of peptides presented in common by these cell types.

NRG-LU001: A phase II trial investigating metformin as a chemo-radio-sensitizer in locally advanced non-small cell lung cancer (NSCLC)



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Several lines of evidence indicated that the anti-diabetic agent metformin may have significant anti-tumor

activity. This is an economical and effective anti-diabetic agent used by more than 120 million patients worldwide and is well-tolerated by non-diabetics too. Metformin is believed to mediate anti-tumor action through blockade of mitochondrial OxPhos complex I and induction of a stage of mild metabolic stress that activates the AMP-activated kinase pathway (AMPK), an enzyme with tumor suppressor activity.

We showed that AMPK is a sensor of both metabolic and genotoxic stress that mediates cell cycle arrest and tumor suppression. Our pre-clinical translational and retrospective clinical studies suggested that targeting metabolism in NSCLC with metformin enhances NSCLC radio-sensitivity and could improve outcomes in locally advanced NSCLC.

NRG-LU001 (NCT02186847) is an NCI-CTEP funded trial that opened to accrual in August 2014. It is designed to examine specifically whether metformin could radio-chemo-sensitize NSCLC in locally advanced stage III (A and B) patients. Its primary outcome is 1 year progression free survival (PFS) and secondary outcomes include: overall survival (OAS); time to local-regional progression (LRP); time to distant metastasis (DM); Chemo-RT toxicity; biospecimen collection for biomarker analysis. This will include: circulating serum and blood cell biomarkers of metformin activity and tumor response and tumor biomarkers such as tumor histology, TP53, LKB1 and K-Ras mutation status.

NRG-LU001 target accrual is 168 patients with 1:1 randomization to either chemo-radiotherapy (CRT) alone vs CRT and metformin of 2000mg daily given only during cytotoxic therapy. This includes concurrent CRT for 6 weeks followed by 6 weeks of consolidation chemo-therapy treatment. Chemotherapy, in both the concurrent and the consolidate phase of this study is the carboplatin-paclitaxel doublet, while patients receive standard chest RT of 60Gy in 30 fractions. Sample size calculations are based on observed 1-year PFS of 50% (RTOG-0617 data) and an expected improvement of 15% with metformin. With 152 analyzable patients this study will have 85% power to detect this improvement. The number is increased to 168 to allow 10% rate of ineligibility.

LU001 is currently open in 52 centers across North America. This is one of the first clinical trials investigating the potential of metabolism modulating agents to enhance chemo-RT responses in locally advanced NSCLC. It is expected to provide initial efficacy results for this agent and assist in the investigation and development of circulating and tumor biomarkers of metformin action and sensitivity. If positive this study will allow us to design rationally future phase III studies to examine definitively the role of metformin in this setting.

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Targeting tumor metabolism to improve radio-sensitivity in non-small cell lung cancer (NSCLC)



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Lung tumor metabolism is severely altered involving enhanced levels of glucose uptake, glycolysis, lipogenesis and protein synthesis. These events are essential for the support of enhanced energy demands and increased need for ribonucleotides, proteins and membrane biogenesis that are required for rapid proliferation.

Combined treatment with cytotoxic therapy and metabolism modulating agents may improve outcomes in NSCLC. We observed that radiotherapy (RT) alone activates the metabolic stress sensor AMP-activated kinase (AMPK) within an Ataxia Telangiectasia Mutated (ATM) – AMPK – p53/p21^{cip1} pathway to mediate the RT-induced G2-M checkpoint and cytotoxicity. Further, inhibition of biosynthetic pathways and energy production through blockade of OxPhos inhibits tumor growth. In human NSCLC cells and xenografts, we combined RT with the anti-diabetic agent metformin, which blocks OxPhos complex I, and showed that metformin activates the ATM-AMPK-p53/p21^{cip1} axis and inhibits the Akt-mTOR-4EBP1 pathway, tumor growth and angiogenesis and induces apoptosis and radio-sensitization.

Based on these observations and supporting retrospective clinical evidence from locally advanced lung cancer patients treated with Chemo-RT, we launched phase II studies combining metformin with chemo-RT. NRG-LU001 (NCT02186847) and OCOG ALMERA (NCT02186847) are on-going randomized phase II studies investigating whether targeting metabolism with metformin can improve progression free survival in stage III NSCLC.

In recent studies we observed that combined treatment with metabolism modulating agents can enhance anti-tumor activity in NSCLC. Combined treatment with Metformin and Salicylate, which activate AMPK through different mechanisms, mediated increased inhibition of clonogenic survival in part through AMPK and suppression of de-novo lipogenesis. Further, in earlier studies we showed that blockade of the cholesterol synthesis pathway with lovastatin (HMG-CoA reductase inhibitor) mediated activation of AMPK, suppression of

the Akt-PI3k pathways and radio-sensitization of NSCLC.

In current studies we observe that lovastatin also activates the ATM-AMPK-p53 axis, inhibit the Akt-mTOR pathway and mediate tumor suppression in an AMPK-dependent manner through suppression of de novo lipogenesis along both the mevalonate and the fatty acid synthesis pathways. Further, in retrospective clinical studies we find that in locally advanced NSCLC patients treated with Chemo-RT, statin treatment is associated with increased survival.

These observations suggest that targeting tumor metabolism is promising in NSCLC to improve outcomes of standard cytotoxic therapy. Completion of on-going trials with metformin will provide the first prospective evidence on this concept. We plan to investigate combinations of well-tolerated metabolism modulating agents that show promising pre-clinical activity in future rolling phase II studies.

OCOG-ALMERA: A phase II trial investigating the ability of metformin to chemo-radio-sensitize and prevent recurrence in locally advanced (LA) non-small cell lung cancer (NSCLC)*



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LA-NSCLC is frequently unresectable and is treated with concurrent chemo-radiotherapy (CRT), which is fairly toxic and provides poor disease control and overall survival. There is an urgent need to develop sensitizers to cytotoxic therapy that could improve the therapeutic ratio in this disease.

Preclinical studies from our group and others demonstrated that the biguanide metformin has activity in lung cancer and sensitizes lung cancer cells and tumors to radiotherapy and chemotherapy. Metformin is known to induce in cells a state of mild metabolic stress through blockade of the mitochondria OxPhos complex I. We showed that metformin alone triggers activation of the ATM-AMP-kinase-p53/p21^{cip1} pathway, inhibition of the radio-resistance Akt-mTOR pathway, radio-sensitizes NSCLC, enhances apoptosis and inhibits angiogenesis.

Metformin is an economical and effective anti-diabetic agent that is well-tolerated by non-diabetics too. Based on our pre-clinical results and additional supporting retrospective data from stage III NSCLC patients treated with chemo-radiotherapy, we launched a phase II clinical trial in locally advanced NSCLC combining metformin with concurrent CRT.